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The expression of sialyltransferases is regulated by the bioavailability and biosynthesis of sialic acids

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ABSTRACT

Glycosylation is the most frequent and important post-translational modification of proteins. It occurs on specific consensus sequences but the final structure of a particular glycan is not coded on the DNA, rather it depends on the expression of the required enzymes and the availability of substrates (activated monosaccharides). Sialic acid (Sia) is the terminal monosaccharide of most glycoproteins or glycolipids (= glycoconjugates) and involved in a variety of function on molecular (e.g. determination of protein stability and half-life) and cellular level (e.g. influenza infection). Sia are synthesized in the cytosol from UDP-GlcNAc by the Roseman-Warren pathway. The key enzyme of this pathway is the UDP-GlcNAc 2-epimerase/ManNAc kinase (GNE). Sia are transferred on glycoconjugates by a family of Golgi-located enzymes, so called sialyltransferases (ST). There are 20 (human) ST known, which all transfer CMP-activated Sia to specific acceptor-sites on glycoconjugates. The regulation of the expression of ST is still not understood. Using a GNE-deficient embryonic stem cell line, which cannot synthesize Sia endogenously and by supplementation of soluble Sia precursors, we present data that the cellular availability of Sia strongly regulates the expression of ST on the level of transcription. In summary, we suggest that the concentration of the donor substrate of sialyltransferases, which can be regarded as a sensor for the environmental conditions of a cell, regulates not only total sialylation, but also the quality of sialylation. This allows a cell to response to altered environmental conditions.

1. Introduction

Posttranslational modifications enrich the repertoire of protein characteristics beyond that is dictated by their primary amino acid sequence (Shental-Bechor and Levy, 2008). Among all post-translational modifications, protein glycosylation is the most abundant protein modification found in nature. It has been estimated that over half of all human proteins are glycosylated (Zafar et al., 2011) supporting the extreme importance of glycosylation. Glycosylation has significant effects on the structure, solubility and stability of the respective proteins. Furthermore, glycans of glycoconjugates are involved in cell-cell and cell-matrix interactions as well as tumor progression and in many pathogen infections (Varki, 2016).

Sialic acid (Sia) represents a family of 9-carbon-containing monosaccharides and is expressed as one out of seven monosaccharides on mammalian glycoproteins (Traving and Schauer, 1998). The typical Sia of mammals is *N*-acetyl neuraminic acid (Neu5Ac). Sia is synthesized from UDP-*N*-acetylglucosamine in the cytosol (Comb and Roseman, 1960) and expressed as terminal monosaccharide on glycans (Fig. 1). The key enzyme of the Sia biosynthesis is the bifunctional GNE (Stäsche et al., 1997). Due to its charge and terminal position, Sia has strong effects on the function of the respective glycoconjugates (e.g. glycoproteins). Sialylation itself occurs in the Golgi and is catalyzed by a family of 20 (human) sialyltransferases (= ST) (Harduin-Lepers et al., 2001), which all use CMP-activated Sia as substrate (Fig. 1).

STs are subdivided into four families according to the

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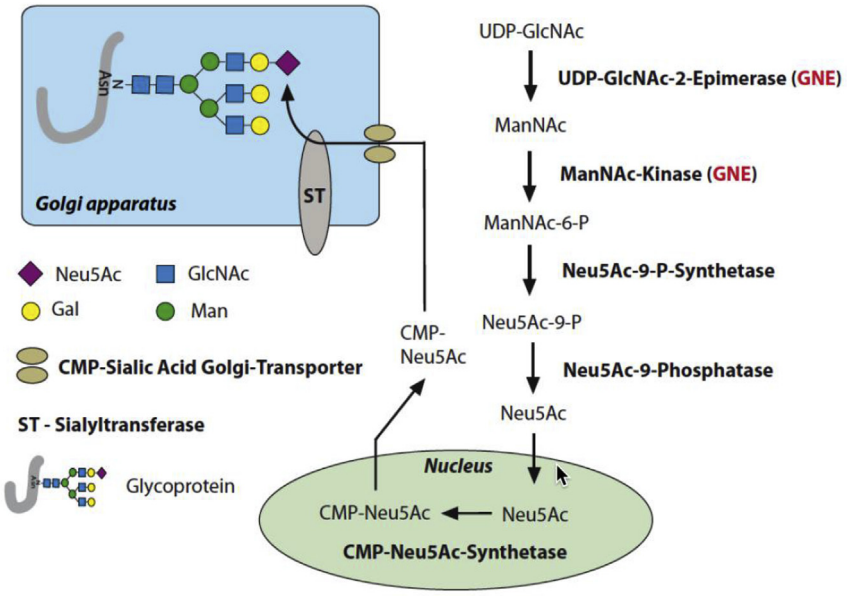

Fig. 1. Schematic representation of the Sia biosynthesis and sialylation of glycoproteins in the Golgi.

carbohydrate linkages they synthesize: β-galactoside α2,3-sialyltransferases (ST3Gal1-6), β-galactoside α2,6-sialyltransferases (ST6Gal1 and 2), GalNAc α2,6-sialyltransferases (ST6GalNAc1-6), and α2,8-sialyltransferases (ST8Sia1-6).

Members of the ST3Gal family transfer Sia from CMP-Sia to terminal galactose residues through 2,3-linkages. Six members of the ST3Gal family have been identified in mouse and humans so far. In contrast, STs of the ST6Gal family transfer Sia from CMP-Sia to terminal galactose residues through 2,6-linkages. To date, only two members of the ST6Gal family, ST6Gal I and II, have been identified in both the mouse and humans. The members of the ST6GalNAc family transfer Sia from CMP-Sia to *N*-acetylgalactosamine residues through 2,6-linkages. Six members of the ST6GalNAc family have been identified in both the mouse and humans.

The last family of STs, the members of the ST8Sia family transfer Sia from CMP-Sia to another terminal Sia residues via 2,8-linkages. Two of them (ST8Sia2 and ST8Si4) are capable to synthesize long homopolymers of Sia, so called polysialic acid (polySia) on the neural cell adhesion molecule NCAM (Finne et al., 1983). To date, six members of the ST8Sia family have been identified in mouse and humans.

Some years ago, we demonstrated by inactivation of the gene for GNE in mice that lack of sialylation is lethal (Schwarzkopf et al., 2002). However, we were able to generate GNE-deficient embryonic stem cells and found by DNA chip arrays that these stem cells differ significantly in their gene expression pattern compared to wildtyp cells (Weidemann et al., 2010). In that study we already found among many other genes ST's to be differential expressed in GNE-deficient stem cells (e.g. ST6Gal1 or ST6GalNAc3), which were not further analyzed at that time. In addition, we could restore sialylation in these cells by feeding GNE-deficient stem cells with the Sia precursor *N*-acetylmannosamine (Weidemann et al., 2010).

In this study we analyzed the expression of all STs in wildtype embryonic stem cells and compared it with embryonic stem cells lacking the GNE. Furthermore we compared ST expression in GNE-deficient embryonic stem cells in the absence or presence of the Sia-precursor ManNAc. We could demonstrate that lack of GNE, which leads to a reduction of sialylation, resulted in an increased expression of ST3Gal5, ST6Gal1 and ST6GalNAc4, but to a decreased expression of ST8Sia1 and to no change of expression of ST8Sia4. By addition of the Sia-precursor ManNAc to GNE-deficient embryonic stem cells, we could reverse this effect. In summary, we were able to demonstrate that the availability of the donor substrates of STs controlled its expression. The more Sia were present, the less STs were expressed and vice versa.

2. Material and methods

2.1. Cell culture

Mouse embryonic stem cells were isolated as described in Schwarzkopf et al. (2002). Cells were cultivated on gelatine-coated flasks in DMEM containing serum replacement (Gibco), 2000 U/ml leukaemia inhibitory factor, 0.1 mM β-mercaptoethanol, 0.2 mM non-essential amino acids, 2 mM L-glutamine and nucleosides. Cells were grown to 80% of confluence and then split 1:2 or 1:3. Cells were passaged every second day and were cultivated in a humidified atmosphere at 37 °C and 5% CO_2. Only cells between passage 10 and 30 were used.

2.2. Quantitative real-time PCR

Total RNA was extracted from embryonic stem cells using TRIzol reagent (Invitrogen). 2 μg RNA were transcribed in cDNA (see RT-PCR above) and 2 μl of a 1:10 dilution of the cDNA was used for quantitative real-time PCR (qRT-PCR). QRT-PCR was performed to quantify the levels of mRNA expression of 4 different genes in GNE +/+ embryonic stem cells or in GNE −/− embryonic stem cells cultivated in SF medium and SFManNAc medium. PCR reaction was performed with iQ.5 Multicolor Real-Time PCR Detection System (BioRad) using the iQ. SYBR. Green Supermix kit (BioRad) according to the manufacture's instruction. The initial denaturation step was 95 °C for 3 min following 40 cycles of 95 °C for 15 s, 55 °C for 30 s and 72 °C for 30 s. The content of selected genes was normalized to the housekeeping gene (HKG) RPL-32. The proper housekeeping gene was evaluated by comparison of housekeeping genes using pair-wise correlations. Calculation was performed using the comparative Ct method according to: